

K 1
33. (Amended) The method of any of claims 24-26, wherein one or more of said inactivated genes are inactivated genes into which at least one nucleotide sequence has been inserted.

34. (Amended) The method of claim 33, wherein a marker gene is inserted into one or more of said inactivated genes.

39. (Amended) The *Shigella* of claim 37, wherein the *Shiga*-toxin gene is *Shiga*-toxin A.

K 2
40. (Amended) The *Shigella* of claim 36 or 37, wherein said *Shigella* is *S. dysenteria* or *S. flexneri*.

41. (Amended) The *Shigella* of claim 37, comprising inactivated ent F, Fep E, Fep C, or Fep D subunit genes of the enterochelin operon.

43. (Amended) The *Shigella* of claim 36 or 37, wherein one or more of said inactivated genes are inactivated genes from which at least one nucleotide sequence has been deleted.

K 3
44. (Amended) The *Shigella* of claim 36 or 37, wherein one or more of said inactivated genes are inactivated genes into which at least one nucleotide sequence has been inserted.

45. (Amended) The *Shigella* of claim 44, wherein a marker gene is inserted into one or more of said inactivated genes.

Please add new claims 47-73 as follows:

47. (New) The method of any of claims 24-26, wherein a marker gene is inserted into each inactivated gene.

48. (New) The *Shigella* of claim 36 or 37, wherein a marker gene is inserted into each inactivated gene.

49. (New) A vaccine comprising the *Shigella* of claim 48 and a pharmaceutically acceptable vehicle.

K4
50. (New) A method for modifying a wild strain of an enteroinvasive *Shigella* to produce a modified strain of *Shigella* that can be used for making a vaccine against the wild strain of *Shigella*, the method comprising inactivating an *icsA* gene of the wild strain of *Shigella* by allelic exchange with a mutagenized *icsA* gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only by means of a transposon inserted into the gene, so that the modified strain of *Shigella* is defective in spread within infected cells and from infected to uninfected cells of the host.

51. (New) The method of claim 50, further comprising inactivating an aerobactin or enterochelin gene of the wild strain of *Shigella* by allelic exchange with a mutagenized aerobactin or enterochelin gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only by means of a transposon inserted into the gene, so that the modified strain of *Shigella* is defective in invading cells of a host.

52. (New) The method of claim 51, further comprising inactivating a *Shiga-toxin* gene of the wild strain of *Shigella* by allelic exchange with a mutagenized *Shiga-toxin* gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only

K4
by means of a transposon inserted into the gene, so that the modified strain of *Shigella* is defective in killing host cells.

53. (New) The method of any of claims 50-52, wherein a marker gene is inserted into one or more of said mutagenized genes.

54. (New) A *Shigella* comprising:

(a) an inactivated *icsA* gene, inactivated by allelic exchange with a mutagenized *icsA* gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only by means of a transposon inserted into the gene; and

(b) an inactivated aerobactin or enterochelin gene, inactivated by allelic exchange with a mutagenized aerobactin or enterochelin gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only by means of a transposon inserted into the gene.

55. (New) The *Shigella* of claim 54, further comprising an inactivated *Shiga-toxin* gene, inactivated by allelic exchange with a mutagenized *Shiga-toxin* gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only by means of a transposon inserted into the gene.

56. (New) The *Shigella* of claim 54 or 55, wherein a marker gene is inserted into one or more of said mutagenized genes.

57. (New) A vaccine comprising the *Shigella* of claim 54 or 55 and a pharmaceutically acceptable vehicle.

58. (New) A method for modifying a wild strain of an enteroinvasive *Shigella* to produce a modified strain of *Shigella* that can be used for making a vaccine against the wild strain of *Shigella* comprising inactivating an *icsA* gene of the wild strain of *Shigella*,

other than only by inactivation by means of a transposon inserted into the gene, to create a modified strain of *Shigella*, so that said modified strain of *Shigella* does not comprise an active *icsA* gene, and said modified strain of *Shigella* is defective in spread within infected cells and from infected to uninfected cells of the host.

K4

59. (New) The method of claim 58, further comprising inactivating an aerobactin or enterochelin gene of the wild strain of *Shigella*, other than only by inactivation by means of a transposon inserted into the gene, so that said modified strain of *Shigella* does not comprise an active copy of the aerobactin or enterochelin gene which was inactivated, and said modified strain of *Shigella* is defective in invading cells of a host.

60. (New) The method of claim 59, further comprising inactivating a *Shiga*-toxin gene of the wild strain of *Shigella*, other than only by inactivation by means of a transposon inserted into the gene, so that said modified strain of *Shigella* does not comprise an active *Shiga*-toxin gene, and said modified strain of *Shigella* is defective in killing host cells.

61. (New) The method of any of claims 58-60, wherein a marker gene is inserted into one or more of said inactivated genes.

62. (New) A *Shigella* comprising:

- (a) an inactivated *icsA* gene, inactivated other than only by means of a transposon inserted into the gene; and
- (b) an inactivated aerobactin or enterochelin gene, inactivated other than only by means of a transposon inserted into the gene;

wherein said *Shigella* does not comprise an active copy of said *icsA* gene and further does not comprise an active copy of said inactivated aerobactin or enterochelin gene.

63. (New) The *Shigella* of claim 62, further comprising an inactivated *Shiga*-toxin gene, inactivated other than only by means of a transposon inserted into the gene; wherein said *Shigella* does not comprise an active copy of said *Shiga*-toxin gene.

64. (New) The *Shigella* of claim 62 or 63, wherein a marker gene is inserted into one or more of said inactivated genes.

K4
65. (New) A vaccine comprising the *Shigella* of claim 62 or 63 and a pharmaceutically acceptable vehicle.

66. (New) A method for modifying a wild strain of an enteroinvasive *Shigella* to produce a modified strain of *Shigella* that can be used for making a vaccine against the wild strain of *Shigella*, the method comprising inactivating an *icsA* gene of the wild strain of *Shigella* by allelic exchange with a mutagenized *icsA* gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only by means of a transposon inserted into the gene, to create a modified strain of *Shigella*, wherein said modified strain of *Shigella* does not comprise an active *icsA* gene, and said modified strain of *Shigella* is defective in spread within infected cells and from infected to uninfected cells of the host.

67. (New) The method of claim 66, further comprising inactivating an aerobactin or enterochelin gene of the wild strain of *Shigella* by allelic exchange with a mutagenized aerobactin or enterochelin gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only by means of a transposon inserted into the

gene, so that said modified strain of *Shigella* does not comprise an active copy of the aerobactin or enterochelin gene which was inactivated, and said modified strain of *Shigella* is defective in invading cells of a host.

68. (New) The method of claim 67, further comprising inactivating a *Shiga-toxin* gene of the wild strain of *Shigella* by allelic exchange with a mutagenized *Shiga-toxin* gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only by means of a transposon inserted into the gene, so that said modified strain of *Shigella* does not comprise an active copy of the *Shiga-toxin* gene, and said modified strain of *Shigella* is defective in killing host cells.

K4
69. (New) The method of any of claims 66-68, wherein a marker gene is inserted into one or more of said mutagenized genes.

70. (New) A *Shigella* comprising:

(a) an inactivated *icsA* gene, inactivated by allelic exchange with a mutagenized *icsA* gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only by means of a transposon inserted into the gene; and

(b) an inactivated aerobactin or enterochelin gene, inactivated by allelic exchange with a mutagenized aerobactin or enterochelin gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only by means of a transposon inserted into the gene;

wherein said *Shigella* does not comprise an active copy of said *icsA* gene and further does not comprise an active copy of said inactivated aerobactin or enterochelin gene.

LAW OFFICES

FINNEGAN, HENDERSON,
FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N.W.
WASHINGTON, DC 20005
202-408-4000